REMARKS

The specification is amended to identify the nucleic acid sequences with SEQ ID NOs.

The application as filed contained two claims 16. The claims are renumbered, and dependencies corrected, to remove the duplication of claim 16. Entry of the foregoing amendment is respectfully requested.

RESPONSE TO RESTRICTION REQUIREMENT

In response to the Restriction Requirement mailed August 22, 2001, Applicant elects, with traverse, the invention of Group X (claims 44-48, 50-54 and 56-60, as renumbered by Examiner and as formally amended herewith), drawn to a method for detecting a known genetic polymorphism in DNA or RNA wherein the detection relies on the adjacent, head-to-tail hybridization of a universal oligonucleotide probe and a polymorphic oligonucleotide probe followed by in situ autoligation to yield an autoligated product. Applicant notes for the record that detection of the autoligated product *optionally* includes detection of a radiolabel present on one or both of the initially separated probes.

Applicant respectfully requests reconsideration and withdrawal or modification of the restriction requirement. It is respectfully submitted that the inventions as claimed can be readily evaluated in one search without placing undue burden on the Examiner. That is, the claims are so interrelated that a search of one group of claims will reveal art to the others.

Were restriction to be effected between and among the claims of Groups I-XI, a separate examination of the claims in these eleven groups would require substantial duplication of work on the part of the U.S. Patent and Trademark Office. Even though some additional consideration would be necessary, the scope of analysis of novelty of all the claims of Groups I-XI would have to be as rigorous as when only the claims of Group X, for example, were being considered by themselves. Clearly, this duplication of effort would not be warranted where these claims of different categories are so interrelated. Further, Applicant submits that for restriction to be effected between and among the claims in Groups I-XI, it would place an undue burden by

requiring payment of ten separate filing fees for examination of the nonelected claims, as well as the added costs associated with prosecuting eleven applications and maintaining eleven patents.

SEQUENCE LISTING

In accordance with 37 C.F.R. §1.821, a computer readable form (CRF) and written Sequence Listing for the above-captioned application are submitted herewith. Applicant respectfully requests entry of same into the specification. A copy of the Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures that accompanied the Restriction Requirement mailed on August 22, 2001, is attached.

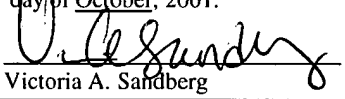
In accordance with 37 C.F.R. §1.821, it is respectfully submitted that the information recorded in computer readable form (CRF) of the Sequence Listing is identical to the written Sequence Listing filed herewith. Additionally, the Sequence Listing submitted herewith contains no new matter.

Applicant notes that despite repeated attempts, the Sequence Listing did not successfully process through Checker Version 3.0. Although the program continued to state that mandatory field data was missing with regard to the title and feature data where the sequence contained "n", careful review of the generated Sequence Listing shows no input errors. Applicant believes all information has been provided as required by 37 C.F.R. §1.821 et seq. If the computer readable form (CRF) of the Sequence Listing is found to be defective in any way, Applicant requests that the error be explained in detail.

CORRECTION OF DRAWINGS

Applicant submits herewith proposed corrected drawings to replace originally filed sheets 4, 5, 7, 8, 11, 12, 13, 16 and 18, and relate to Figures 4, 5, 7, 8, 11, 12, 13, 17, and 19. The proposed corrections identify the sequences contained therein with the assigned SEQ ID NO. These changes are shown in red on the corrected drawings submitted herewith. Approval of the proposed corrected drawings is respectfully requested.

The Examiner is invited to contact Applicant's Representatives, at the below-listed telephone number if prosecution of this application may be assisted thereby.

CERTIFICATE UNDER 37 C.F.R. 1.8:
The undersigned hereby certifies that this paper is being deposited in the United States Postal Service, as first class mail, in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on this 18th day of October, 2001.  Victoria A. Sandberg

October 18, 2001
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Respectfully submitted,

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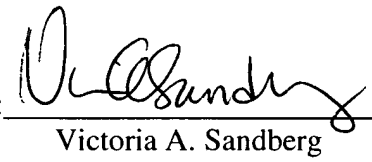
By: 
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APPENDIX A - SPECIFICATION/CLAIM AMENDMENTS
INCLUDING NOTATIONS TO INDICATE CHANGES MADE
Serial No.: 09/483,337
Docket No.: 220.00040101

Amendments to the following are indicated by underlining what has been added and bracketing what has been deleted. Additionally, all amendments have been shaded.

In the Specification

The paragraph beginning at page 9, lines 6-12, has been amended as follows:

Figure 6 shows the time course of autoligation of a hairpin DNA to closed circular dumbbell form at 25 °C (lanes 5-10), with comparison to ligation mediated by T4 DNA ligase in the same sequence (lanes 1-4); complete reaction requires two ligations, the first of which joins two hairpins (creating a nicked dumbbell), and then a second, which closes the dumbbell into circular form; the sequence of the autoligating hairpin is 5'-I-d(TCCAGCGTACTTTTGTACGCTGGATGCA)-p_S-3' (SEQ ID NO:1), and that of the comparison hairpin is d(pTCCAGCGTACTTTTGTACGCTGGATGCA) (SEQ ID NO:2).

The paragraph beginning at page 26, lines 1-12, has been amended as follows:

The stability of the 5'-iodothymidine in comparison to the 5'-tosylthymidine was analyzed by thin layer chromatography under varied conditions. Results showed that the tosylnucleoside in concentrated ammonia (55°C) has a half-life of less than 1 hour, whereas the iodonucleoside has a half-life of about 7 hours. When treated at room temperature for 24 hours (conc. NH_3) the tosylnucleoside is >90% degraded, while the iodonucleoside is <2% degraded. The stability of the iodide in oligonucleotides was also analyzed by reverse-phase HPLC. Chromatograms revealed that the iodide (in the sequence 5'-I-TTCACGAGCCTG) (SEQ ID NO:3) has a half-life of >4 days in conc. NH_3 at 23°C, similar to that of the nucleoside alone. Based on the HPLC analysis we chose the following conditions for deprotection: concentrated ammonia, 55°C for 1 hour, followed by incubation at room temperature for 23 hours, or treatment at room temperature alone for 24 hours. It is anticipated that the iodide would also be stable to rapid deprotection conditions, although this was not explicitly tested.

The paragraph beginning at page 30, lines 4-9, has been amended as follows:

Oligonucleotides containing 5 '-iodo- and 3'-phosphorothioate groups (20 mM) were incubated with 22 mM complementary splint oligomer in a pH 7.0 buffer (50 mM Tris•borate) containing 10 mM $MgCl_2$ at room temperature for 24 hours as described in Example I. The splint sequence used for the 20mer and 45mer DNAs was 5'-d(CTA GTC CAA AGT GCT CGG)(SEQ ID NO:4); for the hairpin sequence no splint was needed. Ligation products were isolated by preparative denaturing polyacrylamide gels.

The paragraph beginning at page 33, lines 2-13, has been amended as follows:

We then investigated the susceptibility of this linkage ([5'-^{32}P]dGATCAGGTp_STTCACGAGCCTG (SEQ ID NO:5), where "s" denotes position of sulfur in phosphorothioate linkage) to different exonuclease enzymes, specifically, the 3'-exonuclease activity of T4 DNA polymerase, snake venom phosphodiesterase (SVPDE) (a different 3' exonuclease), and calf spleen phosphodiesterase (CSPDE), which is a 5'-exonuclease. We found that sulfur causes a significant inhibition of T4 exonuclease activity. The pauses occur at sites one and two nucleotides 3' (prior) to the thioester rather than during the removal of the sulfur-containing nucleotide itself. We estimate the cleavage of the most resistant linkage to be inhibited by a factor of five to tenfold. Since the S-P bond is not expected to be cleaved by this enzyme (products with this enzyme are normally 5'-monophosphates), we surmise that this pause is due to unfavorable interaction of the enzyme with this sulfur, possibly because of the increased bond lengths or relatively poor hydrogen bond accepting ability of the sulfur relative to oxygen.

The paragraph beginning at page 35, lines 5-17, has been amended as follows:

Finally, we examined the effects of one or two 5' bridging sulfur linkages on the thermal stability of DNA-DNA duplexes. This was tested first in the context of a 20mer duplex carrying one sulfur linkage in one strand (d(GAT CAG GTp_ST TCA CGA GCC TG)) (SEQ ID NO:6) and

its unmodified 20mer complement), and a completely unmodified duplex of the same sequence was examined for comparison. Thermal denaturation studies (100 mM Na^+, 10 mM Mg^{2+}) showed that both cases were well-behaved, showing sharp transitions. T_m values were 68.8 °C for the sulfur-containing duplex and 71.5 °C for the unmodified duplex. A second case was then examined with 5'-S linkages in both strands of a duplex, this time using the dumbbell sequences shown in Figure 7). Because of their high stability the denaturation studies were performed under low salt conditions (10 mM Na•PIPES, 1 mM EDTA). The results showed that the thermal stabilities of the modified duplex (T_m = 82.8 °C) and the unmodified one (T_m = 83.3 °C) are essentially the same. Thus, the 5'-bridging sulfur linkage causes very little destabilization of duplexes, at least for the cases studied here.

The paragraph beginning at page 45, lines 22-27, has been amended as follows:

Plasmid pT24 – C3 containing the c-Ha-*ras* 1 activated oncogene mutation at codon 12 (GGC-GTC) and pbc-N1, containing wild type c-Ha-*ras* were obtained from American Type Culture Collection. 300 bp regions including nucleotides – 53 (relative to the transcription initiation site) and + 244 , of normal and activated Ha-*ras* genomic clones were PCR amplified using the primers 5'- GTG-GGG-CAG-GAG-ACC-CTG-TA(SEQ ID NO:7) (sense) and 5'-CCC-TCC–TCT–AGA-GGA-AGC-AG(SEQ ID NO:8) (antisense).

The paragraph beginning at page 56, lines 14-19, has been amended as follows:

Ligated oligonucleotides were characterized by their gel mobility and by electrospray mass spectrometry:

5'dGTG GGC GCC G–pO-TC GGT GT(SEQ ID NO:9) calculated mass 5274.6; found, 5274
5'dGTG GGC GCC G–pS-TC GGT GT(SEQ ID NO:10) calculated mass 5290.6; found, 5290
5'dGTG GGC GCC G–pSe-TC GGT GT(SEQ ID NO:11) calculated mass 5337.6; found, 5337

In the Claims

For convenience, all pending claims are shown below.

1. A nucleotide comprising a phosphoroselenoate group or a phosphorotelluroate group.

2. An oligonucleotide comprising as its 3' end the nucleotide of claim 1.

3. The oligonucleotide of claim 2 comprising as its 5' end a nucleoside comprising a 5' leaving group.

4. An oligonucleotide comprising a plurality of 2'-deoxyribonucleotides and one ribonucleotide, the ribonucleotide comprising a functional group selected from the group consisting of a phosphorothioate group, a phosphoroselenoate group and a phosphorotelluroate group; wherein the oligonucleotide comprises, as its 3' end, the ribonucleotide.

5. A solid support comprising the oligonucleotide of claim 4.

6. An oligonucleotide comprising at least one 5' bridging phosphoroselenoester or phosphorotelluroester.

7. The oligonucleotide of claim 6 comprising at least one deoxyribonucleotide.

8. The oligonucleotide of claim 6 comprising at least one ribonucleotide.

9. The oligonucleotide of claim 6 wherein at least one 5' bridging phosphoroselenoester or phosphorotelluroester forms a bridge between a deoxyribonucleotide and a ribonucleotide.

10. The oligonucleotide of claim 6 that is circular or linear.

11. A nucleic acid duplex comprising the oligonucleotide of claim 2 hybridized to a complementary oligonucleotide.

12. A nucleoside selected from the group consisting of a 5'-deoxy-5'-iodothymidine (5'-I-T), 5'-deoxy-5'-iodo-2'-deoxycytidine (5'-I-dC), 5'-deoxy-5'-iodo-2'-deoxyadenosine (5'-I-dA), 5'-deoxy-5'-iodo-3-deaza-2'-deoxyadenosine (5'-I-3-deaza-dA), 5'-deoxy-5'-iodo-2'-deoxyguanosine (5'-I-dG), 5'-deoxy-5'-iodo-3-deaza-2'-deoxyguanosine (5'-I-3-deaza-dG), 5'-deoxy-5'-iodouracil (5'-I-U), 5'-deoxy-5'-iodocytidine (5'-I-C), 5'-deoxy-5'-iodoadenosine (5'-I-A), 5'-deoxy-5'-iodo-3-deazaadenosine (5'-I-3-deaza-A), 5'-deoxy-5'-iodoguanosine (5'-I-G) and 5'-deoxy-5'-iodo-3-deazaguanosine (5'-I-3-deaza-G), and the phosphoroamidite derivatives thereof.

13. An oligonucleotide comprising as its 5' end a nucleotide derived form the nucleoside of claim 12.

14. An oligonucleotide comprising a plurality of 2'-deoxyribonucleotides and one ribonucleotide, the ribonucleotide comprising a 5' leaving group; wherein the oligonucleotide comprises, as its 5' end, the ribonucleotide.

15. A solid support comprising the oligonucleotide of claim 14.

16. The solid support of claim 15 further comprising an oligonucleotide comprising a plurality of 2'-deoxyribonucleotides and one ribonucleotide, the ribonucleotide comprising a functional group selected from the group consisting of a phosphorothioate group, a phosphoroselenoate group and a phosphorotelluroate group; wherein the oligonucleotide comprises, as its 3' end, the

ribonucleotide

[16] 17. **(Amended)** A solid support comprising at least one oligonucleotide selected from the group consisting of an oligonucleotide comprising a phosphoroselenoate group, an oligonucleotide comprising phosphoroselenoate group, an oligonucleotide comprising a phosphorotelluroate group, and an oligonucleotide comprising a 5' leaving group.

[17] 18. **(Amended)** The solid support of claim [16] 17 comprising an oligonucleotide comprising a 5' leaving group and at least one oligonucleotide selected from the group consisting of an oligonucleotide comprising a phosphoroselenoate group, an oligonucleotide comprising phosphoroselenoate group, an oligonucleotide comprising a phosphorotelluroate group.

[18] 19. **(Amended)** A method for making an oligonucleotide comprising:

binding at least one upstream oligonucleotide and at least one downstream oligonucleotide to a polynucleotide template;

the upstream oligonucleotide comprising, as its 5' end, a nucleoside comprising a 5' leaving group; and

the downstream oligonucleotide comprising, as its 3' end, a nucleoside comprising a 3' phosphoroselenoate or a 3' phosphorotelluroate, wherein the downstream oligonucleotide binds such that it 3' end is substantially adjacent to the 5' end of the upstream oligonucleotide;

to yield an autoligated oligonucleotide product comprising the upstream oligonucleotide ligated to the downstream oligonucleotide.

[19] 20. **(Amended)** The method of claim [18] 19 where one oligonucleotide comprises a fluorescence energy donor group and the other oligonucleotide comprises a fluorescence energy acceptor group, and wherein the presence or absence of the autoligated oligonucleotide product

is accompanied by a detectable change in fluorescence emission of the ligated product compared to the fluorescence energy emissions of the unligated oligonucleotides.

[20]21. **(Amended)** A method for detecting a genetic polymorphism in a target polynucleotide comprising:

providing a mutant polymorphism oligonucleotide probe that is complementary to a region on the target polynucleotide that comprises the genetic polymorphism;

providing a universal oligonucleotide probe capable of binding to the target polynucleotide at a region that is conserved in the analogous wild-type polynucleotide;

wherein one oligonucleotide probe constitutes an upstream oligonucleotide comprising, as its 5' end, a nucleoside comprising a 5' leaving group and the other oligonucleotide probe constitutes a downstream oligonucleotide comprising, as its 3' end, a nucleoside comprising a 3' phosphoroselenoate or a 3' phosphorotelluroate, such that, when both probes are bound to the target polynucleotide, an end of the universal oligonucleotide probe is substantially adjacent to an end of the mutant polymorphism oligonucleotide probe so as to position the 5' leaving group and the 3' phosphoroselenoate or a 3' phosphorotelluroate in close proximity to one another;

contacting the target polynucleotide with the universal oligonucleotide probe and the mutant polymorphism oligonucleotide probe to yield an autoligated oligonucleotide product comprising the universal oligonucleotide probe and the mutant polymorphism probe; and

detecting the presence of the autoligated oligonucleotide product.

[21]22. **(Amended)** The method of claim [20]21 wherein at least one of the mutant polymorphism oligonucleotide probe and the universal oligonucleotide probe comprises a detectable label.

[22]23. **(Amended)** The method of claim [21]22 wherein the detectable label is a radiolabel.

[23] 24. **(Amended)** The method of claim [20] 21 wherein the genetic polymorphism is selected from the group consisting of a single base mutation, a plurality of single base mutations, a deletion, an insertion, and a genetic rearrangement.

[24] 25. **(Amended)** The method of claim [20] 21 wherein the nucleotide position of the genetic polymorphism is not the nucleotide position corresponding to the ligation junction end of the mutant polymorphism probe.

[25] 26. **(Amended)** The method of claim [20] 21 wherein the mutant polymorphism probe is about 3 to about 12 nucleotides in length.

[26] 27. **(Amended)** The method of claim [25] 26 wherein the mutant polymorphism probe is about 3 to about 6 nucleotides in length.

[27] 28. **(Amended)** The method of claim [20] 21 wherein the target polynucleotide is DNA or RNA.

[28] 29. **(Amended)** The method of claim [20] 21 wherein the target polynucleotide is double-stranded or single-stranded.

[29] 30. **(Amended)** The method of claim [20] 21 wherein one oligonucleotide probe comprises a fluorescence energy donor group and the other oligonucleotide comprises a fluorescence energy acceptor group, and wherein the presence or absence of the autoligated oligonucleotide product is accompanied by a detectable change in fluorescence emission of the ligated product compared to the fluorescence emissions of the unligated oligonucleotides.

[30] 31. **(Amended)** A method for determining whether a target polynucleotide contains a

genetic polymorphism comprising:

providing a mutant polymorphism oligonucleotide probe comprising a first fluorescence energy acceptor group, wherein the mutant polymorphism oligonucleotide probe is complementary to a region on the target polynucleotide that comprises the genetic polymorphism;

providing a wild-type polymorphism oligonucleotide probe comprising a second fluorescence energy acceptor group, wherein the wild-type polymorphism oligonucleotide probe is complementary to a region on the analogous wild-type polynucleotide that is analogous to the region comprising the genetic polymorphism;

providing a universal oligonucleotide probe comprising a fluorescence energy donor group, wherein the universal probe is capable of binding to the target polynucleotide at a region that is conserved in the analogous wild-type polynucleotide;

wherein either (i) the universal oligonucleotide probe constitutes an upstream oligonucleotide comprising, as its 5' end, a nucleoside comprising a 5' leaving group and both polymorphism oligonucleotide probes constitute downstream oligonucleotides comprising, as their 3' ends, a nucleoside comprising a 3' functional group selected from the group consisting of a 3' phosphorothioate, a 3' phosphoroselenoate and a 3' phosphorotelluroate; or (ii) both polymorphism oligonucleotide probes constitute upstream oligonucleotides comprising, as their 5' ends, a nucleoside comprising a 5' leaving group and the universal oligonucleotide probe constitutes a downstream oligonucleotide comprising, as its 3' end, a nucleoside comprising a 3' functional group selected from the group consisting of a 3' phosphorothioate, a 3' phosphoroselenoate and a 3' phosphorotelluroate;

such that, when a universal probe and a polymorphism probe are bound to the target polynucleotide, an end of the universal oligonucleotide probe is substantially adjacent to an end of the polymorphism oligonucleotide probe so as to position the 5' leaving group and the 3' functional group in close proximity to one another;

contacting the target polynucleotide with the universal oligonucleotide probe, the mutant

polymorphism oligonucleotide probe and the wild-type polymorphism oligonucleotide probe to yield an autoligated oligonucleotide product comprising the universal oligonucleotide probe either the mutant polymorphism probe or the wild-type polymorphism oligonucleotide probe;

causing the autoligated oligonucleotide product to fluoresce; and

analyzing the fluorescence emission from the autoligated oligonucleotide product to determine whether the autoligated oligonucleotide product comprises the mutant polymorphism probe or the wild-type polymorphism oligonucleotide probe, wherein the presence of the mutant polymorphism probe in the autoligated oligonucleotide product indicates the presence of a genetic polymorphism in the target polynucleotide.

[31] 32. **(Amended)** The method of claim [30] 31 wherein the genetic polymorphism is selected from the group consisting of a single base mutation, a plurality of single base mutations, a deletion, an insertion, and a genetic rearrangement.

[32] 33. **(Amended)** The method of claim [30] 31 wherein the nucleotide position of the genetic polymorphism is not the nucleotide position corresponding to the ligation junction end of the mutant polymorphism probe.

[33] 34. **(Amended)** The method of claim [30] 31 wherein the mutant polymorphism probe is about 3 to about 12 nucleotides in length.

[34] 35. **(Amended)** The method of claim [33] 34 wherein the mutant polymorphism probe is about 3 to about 6 nucleotides in length.

[35] 36. **(Amended)** The method of claim [30] 31 wherein the target polynucleotide is DNA or RNA.

[36] 37. **(Amended)** The method of claim [30] 31 wherein the target polynucleotide is single-stranded or double-stranded.

[37] 38. **(Amended)** A method for detecting a genetic polymorphism in a target polynucleotide comprising:

providing a mutant polymorphism oligonucleotide probe that is complementary to a region on the target polynucleotide that comprises the genetic polymorphism;

providing a universal oligonucleotide probe capable of binding to the target polynucleotide at a region that is conserved in the analogous wild-type polynucleotide;

wherein one oligonucleotide probe constitutes an upstream oligonucleotide comprising, as its 5' end, a nucleoside comprising a 5' leaving group and the other oligonucleotide probe constitutes a downstream oligonucleotide comprising, as its 3' end, a nucleoside comprising a 3' functional group selected from the group consisting of a 3' phosphorothioate, a 3' phosphoroselenoate and a 3' phosphorotelluroate, such that, when both probes are bound to the target polynucleotide, an end of the universal oligonucleotide probe is substantially adjacent to an end of the mutant polymorphism oligonucleotide probe so as to position the 5' leaving group and the 3' functional group in close proximity to one another;

and wherein one oligonucleotide probe comprises a fluorescence energy donor group and the other oligonucleotide comprises a fluorescence energy acceptor group;

contacting the target polynucleotide with the universal oligonucleotide probe and the mutant polymorphism oligonucleotide probe to yield an autoligated oligonucleotide product comprising the universal oligonucleotide probe and the mutant polymorphism probe; and

detecting the presence or absence of the autoligated oligonucleotide product, wherein the presence or absence of the autoligated oligonucleotide product is accompanied by a detectable change in fluorescence emission of the ligated product compared to the fluorescence emissions of the unligated oligonucleotides.

[38] 39. **(Amended)** The method of claim [37] 38 wherein the nucleotide position of the genetic polymorphism is not the nucleotide position corresponding to the ligation junction end of the mutant polymorphism probe.

[39] 40. **(Amended)** The method of claim [37] 38 wherein the mutant polymorphism probe is about 3 to about 12 nucleotides in length.

[40] 41. **(Amended)** The method of claim [39] 40 wherein the mutant polymorphism probe is about 3 to about 6 nucleotides in length.

[41] 42. **(Amended)** The method of claim [37] 38 wherein the target polynucleotide is DNA or RNA.

[42] 43. **(Amended)** The method of claim [37] 38 wherein the target polynucleotide is single-stranded or double-stranded.

[43] 44. **(Amended)** A method for detecting a genetic polymorphism in a target polynucleotide comprising:

providing a mutant polymorphism oligonucleotide probe that is complementary to a region on the target polynucleotide that comprises the genetic polymorphism;

providing a universal oligonucleotide probe capable of binding to the target polynucleotide at a region that is conserved in the analogous wild-type polynucleotide;

wherein one oligonucleotide probe constitutes an upstream oligonucleotide comprising, as its 5' end, a nucleoside comprising a 5' leaving group and the other oligonucleotide probe constitutes a downstream oligonucleotide comprising, as its 3' end, a nucleoside comprising a 3' functional group selected from the group consisting of a 3' phosphorothioate, a 3' phosphoroselenoate and a 3' phosphorotelluroate, such that, when both

probes are bound to the target polynucleotide, an end of the universal oligonucleotide probe is substantially but not directly adjacent to an end of the mutant polymorphism oligonucleotide probe so as to position the 5' leaving group and the 3' functional group in close proximity to one another;

contacting the target polynucleotide with the universal oligonucleotide probe and the mutant polymorphism oligonucleotide probe to yield an autoligated oligonucleotide product comprising the universal oligonucleotide probe and the mutant polymorphism probe; and

detecting the presence of the autoligated oligonucleotide product.

[44] 45. **(Amended)** The method of claim [43] 44 wherein at least one of the mutant polymorphism oligonucleotide probe and the universal oligonucleotide probe comprises a detectable label.

[45] 46. **(Amended)** The method of claim [44] 45 wherein the detectable label is a radiolabel.

[46] 47. **(Amended)** The method of claim [43] 44 wherein the genetic polymorphism is selected from the group consisting of a single base mutation, a plurality of single base mutations, a deletion, an insertion, and a genetic rearrangement.

[47] 48. **(Amended)** The method of claim [43] 44 wherein the nucleotide position of the genetic polymorphism is not the nucleotide position corresponding to the ligation junction end of the mutant polymorphism probe.

[48] 49. **(Amended)** The method of claim [43] 44 where one oligonucleotide comprises a fluorescence energy donor group and the other oligonucleotide comprises a fluorescence energy acceptor group, and wherein the presence or absence of the autoligated oligonucleotide product is accompanied by a detectable change in fluorescence emission of the ligated product compared

to the fluorescence energy emissions of the unligated oligonucleotides.

[49] 50. **(Amended)** A method for detecting a genetic polymorphism in a target polynucleotide comprising:

providing a mutant polymorphism oligonucleotide probe of less than 7 nucleotides in length that is complementary to a region on the target polynucleotide that comprises the genetic polymorphism;

providing a universal oligonucleotide probe capable of binding to the target polynucleotide at a region that is conserved in the analogous wild-type polynucleotide;

wherein one oligonucleotide probe constitutes an upstream oligonucleotide comprising, as its 5' end, a nucleoside comprising a 5' leaving group and the other oligonucleotide probe constitutes a downstream oligonucleotide comprising, as its 3' end, a nucleoside comprising a 3' functional group selected from the group consisting of a 3' phosphorothioate, a 3' phosphoroselenoate and a 3' phosphorotelluroate, such that, when both probes are bound to the target polynucleotide, an end of the universal oligonucleotide probe is substantially adjacent to an end of the mutant polymorphism oligonucleotide probe so as to position the 5' leaving group and the 3' functional in close proximity to one another;

contacting the target polynucleotide with the universal oligonucleotide probe and the mutant polymorphism oligonucleotide probe to yield an autoligated oligonucleotide product comprising the universal oligonucleotide probe and the mutant polymorphism probe; and

detecting the presence of the autoligated oligonucleotide product.

[50] 51. **(Amended)** The method of claim [49] 50 wherein at least one of the mutant polymorphism oligonucleotide probe and the universal oligonucleotide probe comprises a detectable label.

[51] 52. **(Amended)** The method of claim [50] 51 wherein the detectable label is a radiolabel.

[52] 53. **(Amended)** The method of claim [49] 50 wherein the genetic polymorphism is selected from the group consisting of a single base mutation, a plurality of single base mutations, a deletion, an insertion, and a genetic rearrangement.

[53] 54. **(Amended)** The method of claim [49] 50 wherein the nucleotide position of the genetic polymorphism is not the nucleotide position corresponding to the ligation junction end of the mutant polymorphism probe.

[54] 55. **(Amended)** The method of claim [49] 50 where one oligonucleotide comprises a fluorescence energy donor group and the other oligonucleotide comprises a fluorescence energy acceptor group, and wherein the presence or absence of the autoligated oligonucleotide product is accompanied by a detectable change in fluorescence emission of the ligated product compared to the fluorescence energy emissions of the unligated oligonucleotides.

[55] 56. **(Amended)** A method for detecting a genetic polymorphism in a target RNA comprising:

providing a mutant polymorphism oligonucleotide probe that is complementary to a region on the target RNA that comprises the genetic polymorphism;

providing a universal oligonucleotide probe capable of binding to the target RNA at a region that is conserved in the analogous wild-type RNA;

wherein one oligonucleotide probe constitutes an upstream oligonucleotide comprising, as its 5' end, a nucleoside comprising a 5' leaving group and the other oligonucleotide probe constitutes a downstream oligonucleotide comprising, as its 3' end, a nucleoside comprising a 3' functional group selected from the group consisting of a 3' phosphorothioate, a 3' phosphoroselenoate and a 3' phosphorotelluroate, such that, when both probes are bound to the target RNA, an end of the universal oligonucleotide probe is substantially adjacent to an end of the mutant polymorphism oligonucleotide probe so as to

position the 5' leaving group and the 3' functional group in close proximity to one another;

contacting the target RNA with the universal oligonucleotide probe and the mutant polymorphism oligonucleotide probe to yield an autoligated oligonucleotide product comprising the universal oligonucleotide probe and the mutant polymorphism probe; and

detecting the presence of the autoligated oligonucleotide product.

[56] 57. **(Amended)** The method of claim [55] 56 wherein at least one of the mutant polymorphism oligonucleotide probe and the universal oligonucleotide probe comprises a detectable label.

[57] 58. **(Amended)** The method of claim [56] 57 wherein the detectable label is a radiolabel.

[58] 59. **(Amended)** The method of claim [55] 56 wherein the genetic polymorphism is selected from the group consisting of a single base mutation, a plurality of single base mutations, a deletion, an insertion, and a genetic rearrangement.

[59] 60. **(Amended)** The method of claim [55] 56 wherein the nucleotide position is not the nucleotide position corresponding to the ligation junction end of the mutant polymorphism probe.

[60] 61. **(Amended)** The method of claim [55] 56 where one oligonucleotide comprises a fluorescence energy donor group and the other oligonucleotide comprises a fluorescence energy acceptor group, and wherein the presence or absence of the autoligated oligonucleotide product is accompanied by a detectable change in fluorescence emission of the ligated product compared to the fluorescence energy emissions of the unligated oligonucleotides.

[61] 62. **(Amended)** A method for detecting a genetic polymorphism in a target polynucleotide comprising:

providing a mutant polymorphism oligonucleotide probe that is complementary to a region on the target polynucleotide that comprises the genetic polymorphism;

providing a universal oligonucleotide probe capable of binding to the target polynucleotide at a region that is conserved in the analogous wild-type polynucleotide;

wherein one oligonucleotide probe constitutes an upstream oligonucleotide comprising, as its 5' end, a nucleoside comprising a 5' leaving group and the other oligonucleotide probe constitutes a downstream oligonucleotide comprising, as its 3' end, a nucleoside comprising a 3' phosphoroselenoate or a 3' phosphorotelluroate, such that, when both probes are bound to the target polynucleotide, an end of the universal oligonucleotide probe is substantially adjacent to an end of the mutant polymorphism oligonucleotide probe so as to position the 5' leaving group and the 3' phosphoroselenoate or a 3' phosphorotelluroate in close proximity to one another;

contacting the target polynucleotide with the universal oligonucleotide probe and the mutant polymorphism oligonucleotide probe to yield an autoligated oligonucleotide product comprising the universal oligonucleotide probe and the mutant polymorphism probe; and

detecting the presence of the autoligated oligonucleotide product;

wherein the autoligation is reversible by contacting the autoligated oligonucleotide product with silver or mercuric ions.

[62] 63. **(Amended)** A method for detecting a genetic polymorphism in a target polynucleotide comprising:

providing a mutant polymorphism oligonucleotide probe that is complementary to a region on the target polynucleotide that comprises the genetic polymorphism;

providing a universal oligonucleotide probe capable of binding to the target polynucleotide at a region that is conserved in the analogous wild-type polynucleotide;

wherein one oligonucleotide probe constitutes an upstream oligonucleotide comprising, as its 5' end, a 5'-iodopyrene and the other oligonucleotide probe constitutes a downstream oligonucleotide comprising, as its 3' end, a pyrene nucleoside selected from the group consisting of a 3' phosphorothioate, a 3' phosphoroselenoate and a 3' phosphorotelluroate, such that, when both probes are bound to the target polynucleotide, an end of the universal oligonucleotide probe is substantially adjacent to an end of the mutant polymorphism oligonucleotide probe so as to position the 5'-iodopyrene and the 3' pyrene nucleoside in close proximity to one another;

contacting the target polynucleotide with the universal oligonucleotide probe and the mutant polymorphism oligonucleotide probe to yield an autoligated oligonucleotide product comprising the universal oligonucleotide probe, the mutant polymorphism probe, and a pyrene excimer; and

detecting the presence of the autoligated oligonucleotide product using excimers as labels.